Corrections to the Specification

As indicated on page 1

1 **Method for Correcting the Effects of Interdetector Band Broadening.**

2

3 **Related patents**

4 The methods disclosed in this specification will have particular relevance to a variety of patents

5 and co-pending applications by the assignee and inventor. They include the following:

6

7 Steven P. Trainoff and Philip J. Wyatt, ~~Application 10/205,637 filed 24 July 2002,~~ U.S. Patent

8 Number 6,651,009, Issued 18 November 2003, "Method for determining average solution

9 properties of macromolecules by the injection method."

10

As indicated on Page 9 line 3 of the original disclosure in the third paragraph of the section entitled "Detailed description of the invention" which is paragraph 0030 of the application as published

1   When the instrument is used to analyze the eluant of a separation system, such as size exclusion

2   chromatography, SEC, the sample composition and concentration change with time.

3   Furthermore, SEC columns typically dilute the sample concentration so that the second and third

4   terms on the right hand side of Eq. (1) can be neglected compared to the first. Formally this is the

5   case when

6
$$\sout{2A_2cM_w \boxminus 1}\ \underline{2A_2cM_w} \ll 1. \tag{3}$$

7   Typically, for chromatographic separations combined with light scattering instrumentation, the

8   above condition is assumed unless A_2 is known from prior experiment, in which case such value

9   may be used in Eq. 1 directly. In addition, if the separation is performed properly, at each point

10 in time the sample is essentially monodisperse in size, and usually in mass. If the sample is also

11 monodisperse in mass we have the further simplification that

12
$$R(\theta,t) = K^*Mc(t)P(\theta,t). \tag{4}$$

13 Therefore, the light scattering signal should be directly proportional to the concentration signal

14 and the molar mass can be computed by the ratio

15
$$M = \frac{1}{K^*c(t)}\lim_{\theta\to 0} R(\theta,t). \tag{5}$$

16 A common test for the correct operation of a MALS system is to inject a nearly monodisperse

17 sample through a separation column, and then measure both the light scattering and the

18 concentration signals for a monodisperse peak. If normalized, they should overlay exactly at all

19 elution times corresponding to the constancy expressed by Eq. (5). However, if the concentration

20 detector is downstream of the MALS detector, two effects will change the shape of the peak:

21 mixing and diffusion. When this is the case, the peak appears broadened; the center of the peak is

22 suppressed and the "wings" are enhanced. Therefore, when the ratio in Eq. (5) is formed, it will

23 not be constant and the molar mass derived near the peak will be systematically overestimated,

1   while that near the wings will be underestimated. The situation is reversed when the

2   concentration detector is upstream of the MALS detector.

3

As indicated on page 14, line 10 of the original disclosure in the eleventh paragraph of the section entitled "Detailed description of the invention" which is paragraph 0050 of the application as published

1   Once the optimal fit parameters have been determined, there are two ways in which they can be

2   used in the subsequent analyses. One can attempt to "narrow" the concentration measurements

3   by performing a deconvolution of Eq. (10) and attempt thereby, to answer the question: what

4   would a concentration detector have measured if it were coincident with the light scattering

5   detector? Alternatively, one can artificially ~~broadening~~ broaden the light scattering results to

6   answer the conceptual question of what would the light scattering results have been if they were

7   performed downstream coincident with the concentration detector? We will argue that the former

8   method, which has formed the traditional approach, is numerically unstable and often gives

9   unphysical results, while the latter method is numerically stable, but at the expense of reducing

10 the resolution of the measurement. Since the broadening is usually a small correction, the loss of

11 resolution is minimal and the latter method is preferable.

12

As indicated on page 20, line 19 and page 21, line 4 of the original disclosure in the second paragraph of the section entitled "Demonstration of the algorithm" which correspond to paragraphs 0069 and 0070 of the application as published

1   The first step in the algorithm is to choose the broadening model. For the data set in Figure 1, the

2   hybrid model described in Eq.(16) was used. The second step in the algorithm is to choose a

3   monodisperse peak on which to determine the fit parameters. The monomer peak between 16

4   min and 18 min was used. When one forms the χ^2 in Eq. (19), there are four parameters to be

5   determined by nonlinear least squares fitting. An extra parameter was added to account for a

6   possible baseline shift between the two data sets. Therefore the χ^2 that was minimized was

7    $$\chi^2\left(\beta,\tau_0,\sigma,w,x_0\right) = \int_{peak}\left(dRI\left(t\right) - \beta\int_{-\infty}^{\infty} LS\left(t-\tau\right)B\left(\sigma,w,\tau-\tau_0\right)d\tau + x_0\right)^2 dt, \quad (21)$$

8   where, $LS\left(t\right)$ is the 90° light scattering signal as a function of time, and $dRI\left(t\right)$, is the dRI data

9   as a function of time. This model was minimized by using a commercial ~~Marquart~~ Marquardt

10   nonlinear least squares package such as described by D. W. Marquardt in his 1963 article in the

11   Journal of the Society of Industrial and Applied Mathematics, "An algorithm for least squares

12   estimation of nonlinear parameters," volume 11, pages 431 to 441. Note that to perform the

13   broadening correction proscribed by Eq. (20), one does not need β, or x_0, so they will not be

14   presented in the results table below, but they were included to insure that the nonlinear

15   minimization worked correctly.

Parameter	Value
σ'	0.0636 sec
w'	3.178 sec
$\tau_0{}'$	21 sec

16   These results can be interpreted by noting that ~~σ ☐ w, implies~~ $\sigma \ll w$, implying that, as

17   expected, the dominant broadening effect is mixing, not Gaussian diffusion or instrumental

18   broadening. The τ_0 parameter simply indicates that the sample took 21 seconds to traverse

19   between the two instruments. In this context Eq. (20) can be rewritten as

$$LS^b(t) = \int_{-\infty}^{\infty} LS(t-\tau) B\left(\sigma', w', \tau - \tau_0'\right) d\tau. \qquad (22)$$

1

2   Figure 3 shows the broadened light scattering signal **7** computed using Eq. (22) with the fit

3   parameters found in the first step above. The light scattering analysis proceeds as before using

4   Trace **7** instead of the original light scattering data. The results are shown in Trace **6.** There are a

5   number of features to notice. First is that Trace **7** now overlaps Trace **2** at the monomer peak.

6   This shows that model is correct, and that the nonlinear minimization worked properly. The

7   second observation is that the dimer peak, between 14.5 min and 16 min, is now also a plateau as

8   expected. The trimer peak, between 13.5 min and 14.5 min is also flatter, but since it was not

9   baseline separated from its neighboring peaks, the results are less clear. The next observation is

10 that the plateaus are now integral multiples of each other. This is to be expected since the molar

11 mass of the dimer should be exactly twice the mass of the monomer. Similarly, the trimer plateau

12 is now three times that of the monomer.

As indicated on page 6, line 20 of the original disclosure in the new 8th paragraph of the section entitled "Brief description of the drawings" which would appear after paragraph 0023 of the application as published.

This change to the specification references the new Figure. This change and the new figure introduce no new matter.

1 **Brief description of the drawings**

2 Figure 1 shows an uncorrected plot of the molar mass of a BSA sample with the dRI detector

3 downstream of MALS detector.

4 Figure 2 shows the hybrid broadening model fitted to the 90° light scattering trace of a *5ul*

5 injection of 200kD polystyrene in toluene.

6 Figure 3 shows the data of Figure 1 corrected by the method of the invention.

7 Figure 4 shows an uncorrected plot of intrinsic viscosity versus elution volume for a BSA sample

8 with a dRI detector downstream of viscometer.

9 Figure 5 shows the data of Figure 4 corrected by the method of the invention. Trace **11** is the

10 corrected intrinsic viscosity

11 Figure 6 shows an uncorrected plot of the molar mass of a BSA sample with a UV detector

12 upstream of the MALS.

13 Figure 7 shows the data of Figure 6 corrected by the method of the invention.

14 <u>Figure 8 is a graphical representation of the algorithm.</u>

This new paragraph as indicated beginning on page 25, line 5 of the original disclosure preceding the final paragraph of the written description of the specification, which would appear after paragraph 0082 of the application as published.

This change to the specification references the new Figure. This change and the new figure introduce no new matter.

1 The invention is clearly described above in a manner that is understandable to one skilled in the

2 mathematical data analysis arts; however, in order to make the invention more readily

3 understandable, Fig. 8 is presented which graphically illustrates the method of correcting for

4 band broadening using Eq. (19). Figure 8a shows detector signals, $D_1(t)$, **18**, and $D_2(t)$, **19**. The

5 function presented in Eq. (19) can be decomposed into the following steps. First the detector

6 signal with the narrowest peak, **19**, is identified as the signal that will be corrected, *i. e.* will be

7 *broadened.* Signal **19** is then shifted by the interdetector time, τ_2 so it comes into alignment with

8 signal **18**. As shown in Fig. 8b, it is scaled by β_2 so that it is on the same scale/height as the

9 broadest peak, **18**. This newly processed signal peak **20** is then broadened with the

10 model $B(\alpha_{2j}, \tau - \tau_2)$. As shown in Fig. 8c, the broadened data **21** is finally compared to the data

11 of the unprocessed broad peak **18** by computing a χ^2 model which is then minimized to

12 determine optimal values of $\beta_2, \alpha_{2j}, \tau_2$. Figure 8d compares the data after the χ^2 minimization

13 has been completed, *i. e.*, the peaks from the two detectors overlay. It should be stressed that Eq.

14 (19) accomplishes all of these steps simultaneously, as would be obvious to those trained in the

15 art. Although Fig. 8 has been shown in sections for pedagogical reasons, the execution of the

16 least squares process of Eq. (19) is done in a single calculation.

17

18